#12

PATENT
Attorney Docket No.: 15280-325100US
Client Reference No.: E-157-97/0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Susanna M. Rybak *et al.*

Application No.: 09/071,672

Filed: May 1, 1998

For: IMMUNOTOXINS DIRECTED AGAINST MALIGNANT CELLS

Examiner: Amy DeCloux

Art Unit: 1644

DECLARATION OF INVENTORSHIP UNDER 37 C.F.R. § 132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, David M. Goldenberg, hereby declare the following.

I am the named and true inventor of U.S. Patent No. 6,083,477.

To the extent that the subject matter disclosed and claimed in the above-referenced application is also disclosed in said patent, I am not the sole inventor of said subject matter. In particular, I am not the inventor of subject matter relating to onconase immunoconjugates described in Example 2, Example 8, and Table 2 of said patent.

The inventors of said subject matter are Susanna M. Rybak, Dianne L. Newton and David M. Goldenberg.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity f the applicati n or any patent issuing thereon.

Dated: June 7, 2001 By

David M. Goldenberg

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to:

Assistant Commissioner for Patents
Washington, D.C. 20231

On 3 April 2000

TOWNSEND and TOWNSEND and CREW LLP

By: Malinda Wright

PATENT
Attorney Docket No.: 15280-325100US
Client Reference No.: E-157-97/0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Rybak *et al.*

Application No.: 09/071,672

Filed: May 1, 1998

For: IMMUNOTOXINS DIRECTED AGAINST MALIGNANT CELLS

Examiner: Amy DeCloux

Art Unit: 1644

DECLARATION OF SUSANNA RYBAK UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Susanna M. Rybak, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. I currently hold a position with the National Cancer Institute of the National Institutes of Health as a Senior Investigator in the Developmental Therapeutics Program and I am an inventor on the subject application. My curriculum vitae is attached as Exhibit A. I have a Ph.D. from the University of California San Francisco in endocrinology. In 1986 I was appointed an Assistant Professor of Pathology at Harvard Medical School where I began work on a series of human serum ribonucleases. While there I demonstrated that a human serum ribonuclease is a potent inhibitor of protein synthesis and since that time I have been working with ribonucleases (RNase) to develop them as therapeutic agents. I have also authored numerous articles on the subject that are listed on my curriculum vitae.

2. I have reviewed the Office Action mailed on November 2, 1999 in the subject application and am very familiar with the cited art. Our invention relates to a cytotoxic reagent comprising a recognition moiety conjugated to an RNase A protein commonly referred to as "onc" as described in detail in the subject specification. In this Declaration, I will present additional evidence produced by us that further demonstrates the surprising properties of the claimed immunoconjugates. The experimental work described herein was conducted by myself or under my supervision.

3. The data presented in the above-referenced application demonstrated that LL2-ONCONASE® exhibited about 2000 fold greater cytotoxic activity than LL2-EDN. In previous studies (cited in the Office Action mailed November 2, 1999 as Rybak *et al. Tumor Targeting* 1:141-147, 1995) comparing bovine RNase A-containing transferrin immunoconjugates to their onconase-containing counterparts, there was little difference in the relative cytotoxicity of the two reagents. Thus, the markedly enhanced activity of reagents containing onc, *i.e.*, RNase A from *Rana pipiens*, relative to the reagents containing a human RNase was not predicted by us or expected based on our earlier studies.

4. Additional experiments presented herein were performed to show the following: (i) that LL2 conjugated to onc proteins other than ONCONASE® also exhibit markedly enhanced activity and (ii) that an immunoconjugate containing onc linked to an antibody that binds to another B cell marker, CD74, also is surprisingly effective compared to human RNase linked to the same antibody.

The LL2 immunoconjugates that were tested included LL2 conjugated to native onconase (nOnc), LL2 conjugated to the recombinant onc protein *rap*LR1, LL2 conjugated to [Met-(-1)]rOnc (both described PCT Application No: PCT/US97/02588, which is incorporated by reference in the present application) as well as LL2 conjugated to recombinant human EDN (rhEDN) or recombinant human pancreatic RNase (rhpanc). The LL2-[Met-(-1)]rOnc is a control conjugate containing an enzymatically inactive onconase.

The cytotoxicity of LL1-onc immunoconjugates was also additionally evaluated to supplement the data presented in Figure 3 of the present application. LL1 is an IgG1 that specifically bind to the Ii subunit of immature MHC Class II antigens (CD74) and, like LL2, is expressed on human B cells. LL1, also like LL2, is rapidly internalized and catabolized by human B-cell lymphomas (Hansen *et al.*, *Biochem. J.* 320:293-300, 1996 and Shih *et al.*, *Int. J. Cancer* 56:528-545, 1994). The LL1 immunoconjugates tested were LL1 joined to native onconase and LL1 joined to recombinant human EDN.

Cytotoxicity was evaluated by assessing the ability of the immunoconjugates to inhibit protein synthesis. The experiments were performed as follows:

Daudi lymphoma cells (10,000 cell in a 100 μL volume) were aliquoted into each well of a 96-well plate 24 hours before treatment. On the day of treatment, test samples (10 μL) were added to the appropriate wells and the cells were incubated for 3 days at 37°C in a humidified incubator. To determine protein synthesis, the serum-containing media was replaced with 100 μL of serum- and leucine-free RPMI, 0.1 mCi of [^{14}C]leucine (10 μL) was added, and the incubation was continued for 2-4 hrs at 37°C. The cells were then harvested onto glass fiber filters using a PHD cell harvester, the filers were washed with water, dried with ethanol, and the radioactivity was determined in a scintillation counter. Data were obtained in triplicate for each concentration of conjugate tested. The results are presented in Table 1. Activity is reported as an IC_{50}, which is the concentration of test sample that inhibits protein synthesis by 50% as determined from semi-logarithmic plots in which protein synthesis as a % of control (buffer-treated cells) was plotted versus test protein concentration.

5. The data in Table 1 demonstrate that reagents containing the recombinant onconase *rap*LR1 exhibit the same markedly enhanced cytotoxic activity relative to conjugates containing human RNase that was observed with reagents made with the native onconase. The control immunoconjugates containing [Met-(-1)]rOnc, which is a native onconase sequence expressed in bacteria that is inactive because the amino terminal is not translationally processed, did not exhibit enhanced activity relative to the human RNase containing conjugates.

Similarly, the results in Table 1, show that the IC_{50}, the concentration of test sample that inhibits protein synthesis by 50%, is over 100 times greater for the LL1-human RNase

conjugate r lative to the LL1-nOnc conjugate. Thus, LL1-nOnc is over 100 times more effective as a cytotoxic agent relative to the LL1 conjugate made with human RNase.

Table 1. Toxicity of LL2- and LL1-RNase conjugates to Daudi lymphoma cells

Conjugate	IC_{50} (nM)
LL2-nOnc	70
LL2-[Medt-(-1)]rOnc	>50,000
LL2-rapLR1	70
LL2-rhEDN	>50,000
LL2-rhpanc	>100,000
LL1-nOnc	400
LL1-rhEDN	>50,000

6. In summary, we have shown that the surprisingly enhanced cytotoxic activity of onc-containing immunoconjugates relative to human RNase-containing immunoconjugates targeted to B cells is observed with antibodies directed to different B cell markers, and that the enhanced activity is not limited to conjugates made with native onconase.

7. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true. The Exhibit A attached hereto is incorporated herein by reference.

Dated: March 31, 2000

Susanna M. Rybak, Ph.D.

Attachments: Exhibit A

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January 21, 2000

CURRICULUM VITAE

Name: Susanna M. Rybak

Citizenship: United States

Marital Status: Married

Education:

1974 - B.S., University of Massachusetts, Amherst, Massachusetts
1979 - Ph.D., Endocrinology, University of California, San Francisco, California

Postdoctoral Training:

1979-1981 - Research Fellowship, Postdoctoral Fellow, Department of Medicine, Stanford, California
1981-1982 - Research Fellowship, Postdoctoral Fellow, Neurobiology Department, Weizmann Institute of Science, Rehovot, Israel

Employment:

1979-1979 - Lecturer, Department of Biology, Mills College, Oakland, California
1983-1984- Research Associate, Department of Physiology and Biophysics, Harvard Medical School and Dana-Farber Cancer Institute, Boston, Massachusetts
1985-1989 - Research Associate in Pathology, Brigham and Women's Hospital, Boston, Massachusetts
1986-1989 - Assistant Professor of Pathology, Harvard Medical School
1989-1993 - Special Expert, Surgical Neurology Branch, National Institutes of Health, Bethesda, Maryland
1993-1998 Senior Investigator, Laboratory of Biochemical Physiology, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD
1998-date Senior Investigator, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Frederick, MD (GS 14,Tenured Associate Professor, equivalent)

Visiting Appointments:

1973- Visiting Scientist, L'Hospital de Baviere and University of Liege, Liege Belgium
1980- Assistant to the External Examiner, Biochemistry Department, The Chinese University of Hong Kong, Shatin, New Territories Hong Kong
1985 Visiting Scientist, Biochemistry Department, University of Washington, Seattle, Washington
1989 Visiting Scientist, Biogen Corporation, Cambridge, Massachusetts

Honors and Other Special Scientific Recognition:

Honors and Other Special Scientific Recognition:

1965	Who's Who in American Colleges and Universities
1978	Achievement Rewards for College Scientists (ARCS) Foundation Scholarship Awardee
1979	ARCS Foundation Scholarship Awardee
1979-1982	National Institutes of Health Fellowship (3 year award)
1984-1985	Grant for Cancer Research from Elsa U. Pardee Foundation
1997	Japanese Society for the Promotion of Science (JSPS) Award
1995-1998	CRADA (CACR-0290) Continued Investigations of Onconase (3 yr award) from Alfacell Corp., Bloomfield, NJ
1998	Scientific Advisory committee, NMHCC Bio/Technology Conference Division
1999	CRADA (CACR-00746) Development of Antibody RNase conjugates, (3 yr award) from Immunomedics, Morris Plains,NJ
1999	Local Organizing Committee, 5th International Meeting on Ribonuclease, VA, May
1999	CRADA (00754) Development of recombinant RNase fusion protein (3 yr award) from Antisoma Research Laboratories, United Kingdom.
2000	Scientific Advisory committee, NMHCC Bio/Technology Conference Division

Professional Activities:

1996/Spring	Experimental Therapeutics Study Section I, Ad Hoc Reviewer
1996/Fall	Experimental Therapeutics Study Section I, Ad Hoc Reviewer
1995-	Reviewer: Proceedings of the National Academy of Science; Nature Biotechnology; Journal of Biological Chemistry; Journal of the National Cancer Institute; Bioconjugate Chemistry, American J. Pathology, Tumor Targeting,Clinical Cancer Res.J. Immunologial Methods
1997	Fogarty International Center, Fellowship Review Panel
1997-1998	Co-organizer, NCI-FCRDC seminar series for summer students
1998	National Science Foundation, Fellowship Review Panel
1998	Fogarty International Center, Fellowship Review Panel
1998	Editorial Board Journal of the International Society of Tumor Targeting
1998	North Carolina Biotechnology Center, Science & Technology Development Program, Ad Hoc Reviewer

Professional Societies:

1977-Date -	American Society for Cell Biology
1980-Date -	American Association for the Advancement of Science
1994-Date -	International Society of Tumor Targeting
1996-Date-	American Association for Cancer Research

Invited Talks:

Humanization of Immunotoxins, Strategies of Protein Targeting at Royal Free Hospital of London, 1991

Human Immunotoxins, Lederle Laboratories, Pearl River, New York, 1992

Human Immunotoxins, 9th Hammersmith Meeting, Applications of Monoclonal Antibodies in Cancer Therapy, Porto Carras, Greece, 1992

Human Immunotoxins, Gordon Conference, Drug Targeting and Delivery, New Hampshire, 1992

Human Immunotoxins, Designing New Therapeutic Strategies for Cancer and AIDS, PHS Technology Transfer Forum, Bethesda, MD, 1992

Cytotoxic Ribonucleases, NIH Research Day, Bethesda, MD, 1993
Human Immunotoxins, FDA, Bethesda, MD, 1993

Engineered RNase Constructs, 11th Hammersmith Meeting, Applications of Monoclonal Antibodies in Cancer Therapy, Lesvos, Greece, 1994

Immunotoxins Based on Human RNase, 5th Antibody Engineering IBC Conference, San Diego, CA, 1994

RNase Immunofusions, 8th AEK Symposium (German Cancer Society), Heidelberg,FRG

Single Chain Immunofusions Engineered with Human RNases, Exploring and Exploiting Antibody and IG Superfamily Combining Sites, Keystone Symposia, Taos, NM February, 1996

Studies with Targeted Onconase, Immunomedix, Morris Plains, NJ, April, 1996

RNases in the Treatment of Cancer and AIDS, Cooperative Research Center (CRC), Melbourne, Australia, August, 1996

Humanized RNase Based Immunofusions, 10th International Biotechnology Symposium, Sydney, Australia, August, 1996

Immunotoxins, CRC for Diagnostic Technologies, Brisbane, Australia, August, 1996

From an Angiogenic Factor to an Immunotoxin, Ribonuclease Minisymposium, Bloomfield, NJ, October, 1996

RNase Based Immunotoxins for Cancer Therapy, Department of Surgery, Keio University School of Medicine, Tokyo, Japan,March, 1997

Targeting Cancer Cells with RNase Chimeric Proteins, Department of Bioengineering, Okayama University, Okayama, Japan, March, 1997

Ribonuclease Based Immunotoxins for the Treatment of Lymphomas. Monoclonal Antibodies in Clinical Oncology, Santorini, Greece, May, 1997

Ribonuclease-Based Immunotoxins: Functional Studies. 8th Antibody Engineering IBC Conference, San Diego, CA, December, 1997

RNase-Based Therapeutics for Cancer and AIDS, Monoclonal Antibodies in Clinical Oncology, Santorini, Greece, May, 1998

Ribonuclease-sFv Fusion Protein, Antibody Engineering and Expression, Arlington, VA, June, 1998

Engineered and Human Ribonuclease-based Immunotoxins, 9th Antibody Engineering IBC Conference, San Diego, CA, December, 1998

RNase-based Anti-B Cell Therapeutics, 5th International Conference on Ribonuclease, Airlie Conference Center, Warrenton, VA, May 1999

Anti-angiogenic RNases, In Vitro Transformation Meeting, July 24-25, 1999, Cork, Ireland

Antibody enzyme fusions, Antibody Engineering Workshop, November 3-4, 1999, Heidelberg, FRG.

Developments in RNase-based Therapeutics, King Fredrico II University, November 8, 1999 Naples, IT

Antibody enzyme fusions , 10th Anniversary Antibody Engineering, December 6-9, 1999, San Diego, CA

Phage mediated biological delivery of RNase to tumors, Gordon Conference: Drug Delivery in Medicine, February 20-25, 2000, Ventura, CA

Anti-CD22 RNase conjugates, International Conference on Advances in Cancer Immunotherapy March 2-4, 2000, Princeton, NJ

RNase-based therapeutics: an update. Advances in the application of monoclonal antibodies in clinical oncology, May31-June2,2000, Samos, Greece.

Expression of Immunoenzymes in the milk of Transgenic animals. Cambridge Healthtech Institute, Recombinant Antibody Conference, June 5-6,2000, Baltimore, MD.

Drug Development Status:

1998RFB4-Onconase (NSC 703939), Anti-B cell Immunoenzyme, approved National Cancer Institute, DN Stage II, formulation, pharmacology and primate toxicology

Social Security No.: 029-32-2650

Present Address: 7411B Round Hill Road
Frederick, MD 21702

Publications:

1.Ramachandran, J., Farmer, S.W., Liles (Rybak), S.M., and Li, C.H.: Comparison of the steroidogenic and melanotropic activities of corticotropin, melanotropin and analogs with their lipolytic activities in rat and rabbit adipocytes. Biochim. Biophys. Acta. 428:347-354, 1976.

2.Ramachandran, J., Kong, Y.C., and Liles (Rybak), S.M.: Effects of ACTH and its O-nitrophenyl sulphenyl derivative and adrenocortical function in vivo. Acta Endocrinol. 82:587-599, 1976.

3.Ramachandran, J., Rao, A.J., and Liles (Rybak), S.M.: Studies on the trophic action of ACTH. Ann. NY. Acad. Sci. 297:336-348, 1977.

4.Liles (Rybak), S.M. and Ramachandran, J.: Regulation of 3 beta-hydroxysteroid dehydrogenase isomerase activity in adrenocortical cell cultures by ACTH. Biochem. Biophys. Res. Commun. 79:226-233, 1977.

5.Rybak, S.M. and Ramachandran, J.: Primary culture of normal rat adrenocortical cells. I: Culture conditions for optimal growth and function. In Vitro 79:599-604, 1981.

6.Rybak, S.M. and Ramachandran, J.: Primary culture of normal rat adrenocortical cells. II: Quantitation of steroid dehydrogenase stain. In Vitro 17:605-611, 1981.

7.Rybak, S.M. and Ramachandran, J.: Comparison of the structural features of corticotropin required for stimulation of steroidogenesis and cAMP production in rat and rabbit adrenocortical cells. Int. J. Pept. Protein Res. 18:148-153, 1981.

8.Rybak, S.M. and Stockdate, F.E.: The mitogenic effects of lithium chloride in BALB/c-3T3 fibroblasts and Madin-Darby canine kidney epithelial cells. Exp. Cell. Res. 136: 263-270, 1981.

9.Rybak, S.M. and Ramachandran, J.: Mechanism of induction of 3ß-hydroxysteroid dehydrogenase isomerase activity in rat adrenocortical cells by corticotropin. Endocrinology 111:427-433, 1982.

10.Ginzburg, I., Rybak, S.M., Kimhi, Y., and Littauer, U.Z.: Biphasic regulation by dibutyryl cyclic-AMP of tubulin and actin mRNA levels in neuroblastoma cells. Proc. Natl. Acad. Sci. USA. 80:4243-4247, 1983.

11.Rybak, S.M., Ginzburg, I., and Yavin, E.: Gangliosides stimulate neurite outgrowth and induce tubulin mRNA accumulation in neural cells. Biochem. Biophys. Res. Commun. 116:974-980, 1983.

12.Ginzburg, I., Scherson, T., Rybak, S.M., Kimhi, Y., Neuman, D., Schwartz, M. and Littauer, U.A.: Expression of mRNA for microtubule proteins in the developing nervous system. In: Cold Spring Harbor Symposia on Quantitative biology. Cold Spring Harbor Press, New York, 783, 1983.

13.Rheinwald, J.G., O'Connell, T.M., Connell, N.D., Rybak, S.M., Allen-Hoffman, B.L., LaRocca, P.F., Wu, Y.J., and Rehwoldt, S.M.: Expression of specific keratin subsets and vimentin in normal human epithelial cells - function of cell type and conditions of growth during serial culture. Arranged by Arnold J. Levine: Cold Spring Harbor Conferences on Cell Proliferation and Cancer: The Cancer Cell. Cold Spring Harbor, New York, Cold Spring Harbor Press, 1983, 217-227.

14.Littauer, U.Z., Zutra, A., Rybak, S.M. and Ginzburg, I.: The expressionof tubulin and various enzyme activities during neuroblastoma differentiation. Prog. Clin. Biol. Res. 175: 193-208, 1985.

15.Lobb, R.R., Rybak, S.M., St. Clair, D.K., and Fett, J.W.: Lysates of two established human tumor lines contain heparin-binding growth factors related to bovine acidic brain fibroblast growth factor. Biochem. Biophys. Res. Commun. 139:861-867, 1986.

16.Rybak, S.M., Fett, J.W., Yao, Q.Z., and Vallee, B.L.: Angiogenin mRNA in human tumor and normal cells. Biochem. Biophys. Res. Commun. 146:1240-1248, 1987.

17.St. Clair, D.K., Rybak, S.M., Riordan, J.F., and Vallee, B.L.: Angiogenin abolishes cell-free protein synthesis by specific ribonucleolytic inactivation of ribosomes. Proc. Natl. Acad. Sci. USA. 84:8330-8334, 1987.

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19.Kurachi, K., Rybak, S.M., Fett, J.W., Shapiro, R., Strydom, D.J., Olson, K.A., Riordan, J.F., Davie, E.W., and Vallee, B.L.: Expression of human angiogenin in cultured baby hamster kidney cells. Biochemistry 27:6557-6562, 1988.

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24.Rybak, S.M. and Youle, R.J.: Clinical use of immunotoxins: Monoclonal antibodies conjugated to protein toxins. Immunology and Allergy Clinics of North America. 11:2 359-380, 1991.

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Mikulski, S.M., Ardelt, W., Riggs, C., Kung, H.F., and Longo, D.L.: Vincristine cytotoxicity is enhanced by Onconase, an anti-tumor RNase even in the presence of mdr1 expression. J. Natl. Cancer Inst.88:747-753, 1996.

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47.Cara, A., Rybak, S.M., Newton, D.L., Crowley, R., Rottschafer, S.E., Reitz, M.S. And Gusella, G.L. Complete Inhibition of HIV-1 replication by combined expression of gag dominant negative mutant and a human ribonuclease in a tightly controlled HIV-1 inducible vector. Gene Therapy 5: 65-75, 1998.

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